SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. cPLA\(_2\varepsilon\) overexpression induces tubulation of MHC-I compartment.
HeLa cells were trasfected with either GFP-cPLA\(_2\varepsilon\), fixed, labeled with antibodies against endogenously-expressed MHC-I (A), TfR (B), EEA1 (C), Rab5 (D), Lamp1 (E) and analyzed under confocal microscope. Arrows in panel A indicate strong tubulation of MHC-I-positive membranes in cell, which overexpress cPLA\(_2\varepsilon\), while patterns of other markers did not exhibit significant alterations in cPLA\(_2\varepsilon\)-positive cell (B-E). Scale bar: 5\(\mu\)m (A-E).

Supplementary figure 2. Analysis of cPLA\(_2\varepsilon\) co-localization with markers of different endocytic pathways.
HeLa cells were grown on cover-slips and transfected with mCherry-tagged cPLA\(_2\varepsilon\) alone (A-C) or cotransfected with mCherry-tagged cPLA\(_2\varepsilon\) and ARF6\(^{Q67L}\) (D). After 24h cells were incubated for 30 minutes at 37°C with medium containing anti-MHC-I antibody (A), Alexa 488- transferrin (B), or Alexa 488-EGF (C). At the end of the incubation the cells were washed, fixed and processed for confocal microscopy. A. Colocalization of cPLA\(_2\varepsilon\) with uptaken MHC-I was detected both in tubular (arrows) and vesicular (arrowheads) endocytic structures. B. Internalized transferrin did not exhibit substantial overlap with tubular (arrows) or vesicular (arrowheads) cPLA\(_2\varepsilon\)-positive structures. C. cPLA\(_2\varepsilon\)-positive tubular (arrows) or vesicular (arrowheads) structures did not receive internalized EGF. D. Arrows show large endocytic structures decorated by both cPLA\(_2\varepsilon\) and ARF6\(^{Q67L}\). Scale bar: 4\(\mu\)m (A-D).

Supplementary figure 3. cPLA\(_2\varepsilon\) silencing affects MHC-I recycling.
A. Control and cPLA\(_2\varepsilon\)-silenced HeLa cells were labeled at 4°C with anti MHC-I antibody and incubated with for 20 min at 37°C to stimulate the antibody uptake. Then the cells were washed with acid buffer and fixed directly (A, upper row). Alternatively, the cells were incubated for 30 minutes at 37°C to allow MHC-I recycling (A, lower row).
and subjected to an acid wash before fixation. In this way the IF analysis revealed only the intracellular MHC-I pool, which remained significantly higher in cPLA₂ε-silenced cells. B. Chart in panel B shows normalized fluorescent intensity of MHC-I (average ± SD, n=50 cells) to remain higher in cPLA₂ε-silenced cells (*** p<0.001 according t-test). Scale bar: 7µm (A).

**Supplementary figure 4. Depletion of cPLA₂ε has no significant impact on trafficking routes other than CIE.**

HeLa cells grown on coverslips were treated with scramble (indicated as Control) or cPLA₂ε SiRNA (indicated as RNAi). After 96 h of treatment, a set of covers slips were fixed and stained for anti M6PR (A) or caveolin (B). Another set of cover slips were incubated at 37°C with medium containing EGF for 30 minutes (C) or Alexa 546-Transferrin for 10 and 20 minutes (D). At the end of the incubation, cells were fixed and stained for anti EGFR (C) or simply processed for IF analysis (D). Scale bar: 8 µm (A-D).
Suppl. Fig. 3 Capestrano et al.

A

Control

RNAi

0 min

30 min

B

Normalized intensity %

Control

RNAi

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Suppl. Fig. 4 Capestrano et al.